REMARKS

The Invention

The present invention relates to methods for increasing the efficiency of the transfection of cycling cells. The methods involve synchronizing cells with electromagnetic radiation and transfecting the cells within about one cell cycle with a nucleic acid. The electromagnetic radiation is selected from Gamma rays, X-rays, or ultraviolet rays, and the efficiency of transfection is increased over cells not contacted with the electromagnetic radiation.

Status of the Claims

Applicants wish to thank Examiner Woitach for extending the courtesy of the telephonic interview held on February 5, 2004 with Applicants' representatives Carol A. Fang and Eugenia Garrett-Wackowski. During this interview, a number of issues were clarified which have helped Applicants to more fully address the concerns of the Examiner. Applicants thank Examiner Woitach for his time.

After entry of this amendment, claims 1-5, 7-10, 12, and 46 are pending. In accordance with the Examiner's suggestion, Applicants have amended claim 1 to delete the recitation "at least about fivefold," and respectfully request entry of this amendment. Support for this amendment can be found throughout the specification and claims as originally filed. Thus, no new matter has been introduced by this amendment and this amendment does not necessitate a new search. Accordingly, Applicants respectfully request entry of this amendment.

A clean copy of the claims is provided in Appendix A for the Examiner's convenience.

In the Office Action mailed November 19, 2004, the pending claims were rejected under 35 U.S.C. § 112, first paragraph and under 35 U.S.C. § 103(a). Each of these rejections is addressed below.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-7, 7-10, 12, and 46 were initially rejected under 35 U.S.C. §112, first paragraph, as allegedly nonenabled for increasing the efficiency of transfection at least about fivefold.

As discussed during the interview of February 5, 2004, and in accordance with the Examiner's suggestion, the claims have been amended to clarify the scope of the invention. More particularly, the claims have been amended to recite contacting cells with electromagnetic radiation and transfecting the cells with a nucleic acid to increase the efficiency of transfection over cells not contacted with the electromagnetic radiation. The specification provides ample guidance for one of skill in the art to practice the presently claimed methods of increasing transfection efficiency. For example, Example 11 describes methods of increasing transfection efficiency using x-ray irradiation (*see, e.g.*, specification at page 54, line 8 to page 55, line 8). Moreover, as the Examiner noted during the interview, it is known to those of skill in the art that various forms of electromagnetic radiation exert their biological effects through the same mechanism and biological pathway. Thus, one of skill in the art would appreciate that other forms of electromagnetic radiation (*i.e.*, gamma rays and ultraviolet rays), would be used in a manner similar to x-rays.

Therefore, Applicants respectfully submit that the claims are fully enabled. Accordingly, Applicants urge the Examiner to withdraw the rejection under 35 U.S.C. § 112, first paragraph.

Rejections Under 35 U.S.C. § 103

The claims were initially rejected, in various combinations, under 35 U.S.C. § 103(a) over a number of different references. Applicants respectfully traverse each of the § 103 obviousness rejections. As set forth in M.P.E.P. § 2143:

> [t]o establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference

> or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim elements. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

As discussed with the Examiner and explained herein below in connection with each of the § 103(a) obviousness rejections, Applicants assert that a prima facie case of obviousness has not been established. First, the cited references do not teach or suggest all of the claim limitations. Moreover, one of skill in the art would have no motivation to combine the cited references. Finally, even if one of skill in the art were to combine the teachings of the cited references, there would be no reasonable expectation of success in arriving at the claimed methods of increasing the efficiency of cell transfection.

The present invention is directed to methods for increasing the efficiency of the transfection of cycling cells by synchronizing cells with electromagnetic radiation (*e.g.*, gamma rays, X-rays, or ultraviolet rays) and transfecting the cells within about one cell cycle with a nucleic acid. The efficiency of transfection is increased over cells not contacted with the electromagnetic radiation.

1. Rejection of claims 1-5, 7-9, 12, and 46 over Yorifuji et al. in view of Spang-Thomsen et al.

Claims 1-5, 7-9, 12, and 46 were initially rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Yorifuji *et al.* in view of Spang-Thomsen *et al.* In making this rejection, the Examiner acknowledges that Yorifuji *et al.* do not teach the use of electromagnetic radiation to synchronize cells, but alleges that Yorifuji *et al.* disclose that synchronizing cells with chemical agents leads to increased transfection efficiency, alleges that Spang-Thomsen *et al.* disclose that cells can be synchronized with x-rays, and concludes that it would have been obvious to synchronize cells with electromagnetic radiation to increase transfection efficiency. Applicants respectfully traverse.

The cited references do not disclose all of the elements of the claimed invention

Yorifuji *et al.* and Spang-Thomsen *et al.* do not disclose all of the elements of the claimed methods of increasing transfection efficiency. Yorifuji *et al.* disclose only that treatment of cells with one of two ***chemical*** agents, *i.e.*, hydroxyurea and aphidicolin, leads to greater transformation efficiency when the cells are transfected with a linearized plasmid. As acknowledged by the Examiner and discussed during the interview, Yorifuji *et al.* does not disclose or suggest use of electromagnetic radiation to increase transfection efficiency and Spang-Thomsen *et al.* does not remedy the deficiency in Yorifuji *et al.* Spang-Thomsen *et al.* describe experiments designed to lead to improved x-ray irradiation therapy to kill tumor cells and observes that a small percentage (*i.e.*, a maximum of 20 %) of x-ray irradiated tumor cells accumulate at the G_2/M phase of the cell cycle after x-ray treatment. Spang-Thomsen *et al.* does not disclose or suggest transfection of the x-ray irradiated cells or that transfection efficiency would be improved by x-ray irradiation of cells. Therefore, Yorifuji *et al.* and Spang-Thomsen *et al.*, alone or in combination, fail to disclose or suggest all of the elements of the presently claimed methods of improving transfection efficiency.

One of skill in the art would have no motivation to combine the cited references

As discussed above, Yorifuji *et al.* describe use of a chemical agent to synchronize cells and improve transfection efficiency while Spang-Thomsen *et al.* observe that X-ray irradiation to kill tumor cells leads to accumulation of at most 20% of the cells at the G_2/M phase of the cell cycle. Neither of the references discloses or suggests using electromagnetic irradiation in a method to improve transfection efficiency. As discussed during the interview, the cited references alone and in combination are devoid of any nexus between accumulation of 20% of x-ray treated cells at a particular cell cycle phase and an increase in transfection efficiency and thus provide no motivation for one of skill in the art to combine the references.

Moreover, as discussed during the interview, one of skill in the art would appreciate that chemical agents and electromagnetic radiation exert their effects through different

biological mechanisms and, thus, would not be motivated to modify a chemical agent-based method of synchronizing cells for an electromagnetic radiation-based method of synchronizing cells without a specific suggestion to do so. Therefore, one of skill in the art would not be motivated to modify the disclosure of Yorifuji *et al.* and substitute electromagnetic irradiation for treatment with a chemical agent.

One of skill in the art would have no reasonable expectation of success in arriving at the claimed methods by combining the cited references

Even if the cited references were combined, one of skill in the art would have no reasonable expectation of success in arriving at the claimed methods of increasing transfection efficiency. As discussed with the Examiner, the cited references demonstrate that, the use of one chemical agent to increase in transfection efficiency cannot easily be extrapolated to the use of other chemical agents or other methods to increase transfection efficiency.

For example, Son *et al.*, cited in connection with the 35 U.S.C. § 103(a) rejection of claim 10, explicitly sets forth experiments demonstrating that even structurally related chemical agents do not sensitize cells to transfection. In particular, Son *et al.* demonstrate that only one of eight chemical agents, *i.e.*, cisplatin, was able to sensitize cells to transfection (*see, e.g.*, Figure 4 and page 12671, col. 2, lines 13-14). Son *et al.* specifically set forth that other chemical agents including transplatin (a geometric isomer of cisplatin), carboplatin (an agent with similar anti-tumor effects as cisplatin), MTX, etoposide, vincristine, and Ara-C, all had no effect in sensitizing cells to transfection (*see, e.g.*, page 12671, col. 2, lines 16-20). Therefore, based on the disclosure of Son *et al.*, one of skill in the art would appreciate that even related chemical agents do not have similar effects on the transfection efficiency and one of skill would not expect that modifying the teachings of Yorifuji *et al.* to use other methods of synchronizing cells, *i.e.*, other chemical agents or electromagnetic radiation, would lead to increased transfection efficiency.

Absent a teaching or suggestion to use electromagnetic radiation to improve transfection efficiency as disclosed and claimed in the present invention, the present invention is

non-obvious and, thus, patentable. Accordingly, Applicants urge the Examiner to withdraw this rejection under 35 U.S.C. § 103(a).

2. *Rejection of claim 10 over Yorifuji et al. in view of Spang-Thomsen et al., further in view of Son et al.*

Claim 10 was initially rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Yorifuji *et al.* in view of Spang-Thomsen *et al.* as applied to claims 1-5, 7-9, 12, and 46, and further in view of Son *et al.* In making the rejection, the Examiner alleges that Son *et al.* teach transformation of a cell with a lipid-nucleic acid particle. Applicants respectfully traverse.

As explained above and discussed with the Examiner, one of skill in the art would not have been motivated to combine the teachings of Yorifuji *et al.* and Spang-Thomsen *et al.* Even if one of skill in the art were to combine Yorifuji *et al.* with Spang-Thomsen *et al.*, the combination would not lead to the claimed invention because one of skill in the art would not expect that substitution of a chemical agent for electromagnetic radiation would improve transfection efficiency. Son *et al.* do not remedy the deficiencies of Yorifuji *et al.* and Spang-Thomsen *et al.*

Son *et al.* disclose a DNA-liposome ***complex*** prepared by mixing DNA with liposomes (see, page 12669, col. 2). In the DNA-liposome complex of Son *et al.*, the DNA is not fully encapsulated in the liposome. In contrast, to Son *et al.*, claim 10 recites "wherein said nucleic acid is fully encapsulated in a lipid-nucleic acid particle." The specification at page 26, lines 21-25 describes the encapsulated, nuclease resistant lipid-DNA particles as claimed in the present invention, and clearly they are different from the DNA-liposome complex of Son *et al.*

Moreover, as noted by the Examiner during the interview and discussed in detail above, Son *et al.* describe using a variety of structurally and mechanistically unrelated chemical agents to sensitize cells to transfection with the DNA-liposome complexes. As specifically set forth in Son *et al.*, only one of the agents, *i.e.*, cisplatin, was effective in sensitizing cells to such transfection. The Examiner agreed that, in view of the teachings of Son *et al.*, one of skill in the art would not have been motivated to use other chemical agents, much less use electromagnetic

radiation to increase transfection efficiency. In addition, the Examiner agreed that one of skill in the art would not have expected that modifying the disclosure of Yorifuji *et al.* would lead to the claimed methods of increasing transfection efficiency.

Absent a teaching or suggestion to use electromagnetic radiation to synchronize cells to improve transfection efficiency as disclosed and claimed in the present invention, the present invention is non-obvious and, thus, patentable. In view of the foregoing, Applicants respectfully submit that the presently claimed methods of increasing transfection efficiency are nonobvious and thus patentable. Accordingly, Applicants urge the Examiner to withdraw this rejection under 35 U.S.C. § 103(a).

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at 415-576-0200.

Respectfully submitted,

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APPENDIX A

PENDING CLAIMS

1. (Currently Amended) A method of increasing the efficiency of transfection of cycling cells sensitive to electromagnetic radiation, comprising:

synchronizing said cells by contacting said cells with electromagnetic radiation, wherein said electromagnetic radiation is a member selected from the group consisting of: Gamma rays, X-rays, and ultraviolet rays, and

transfecting said cells within about one cell cycle with a nucleic acid that encodes a desired gene product,

wherein said efficiency of transfection is increased over cells not contacted with said electromagnetic radiation.

2. (Previously presented) A method of claim 1 wherein said electromagnetic radiation synchronizes cells at a stage of the cell cycle when the nuclear membrane is substantially degraded.

3. (Previously presented) A method of claim 1 wherein said electromagnetic radiation synchronizes cells at late S phase.

4. (Previously presented) A method of claim 1 wherein said electromagnetic radiation synchronizes cells at the G2/M phase boundary.

5. (Previously presented) A method of claim 1 wherein said electromagnetic radiation synchronizes cells at a stage other than M phase, and the nucleic acid accumulates in cells that have cycled to the G2/M phase boundary.

6. (Canceled)

7. (Previously presented) A method of claim 1 wherein said gene product is foreign to said cells.

8. (Previously presented) A method of claim 1 wherein said gene product is toxic to said cells.

9. (Previously presented) A method of claim 8 wherein said gene product induces apoptosis.

10. (Previously presented) A method of claim 1 wherein said nucleic acid is fully encapsulated in a lipid-nucleic acid particle.

11. (Canceled)

12. (Previously presented) The method of claim 1, wherein said electromagnetic radiation is X-rays.

13-45. (Canceled)

46. (Previously presented) The method of claim 1, wherein said cells are present within a mammal.